Antigen Antibody reactions

a reaction that occurs when an **antigen** combines with its specific **antibody** to produce an **immune complex**

⬇

In vitro tests that detects formed immune complexes

⬇

Serological tests

⬇

Detection of **unknown Ag or Ab**

⬇

Diagnosis of microbial infection

Serological Reactions / Tests

1-Agglutination

2.Precipitation

3.Enzyme linked immunosorbant Assay (ELISA)

4.Immunofluorescence (IF)

5.Radioimmunoassay (RIA)

6.Immunoblotting

7-Neutralization

8-Complement Fixation

Agglutination:Direct &Indirect

I-Direct Agglutination

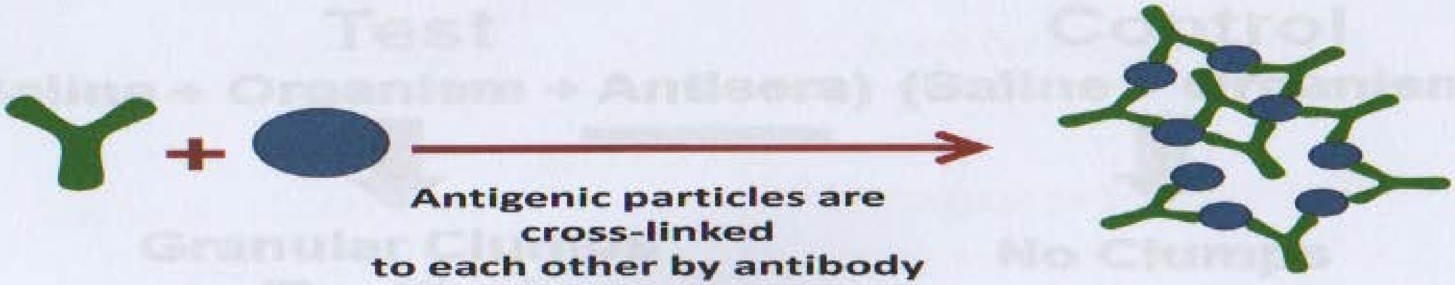
agglutination = (visible clumping) of the antigen

Agglutinin
IgM (main)
IgG

+

Agglutinogen
Insoluble Or
particulate(large)

= **Agglutination**



Antigenic particles are cross-linked to each other by antibody

large lattice of Ag-Ab complex

Visible Deposit

Direct Agglutination tests

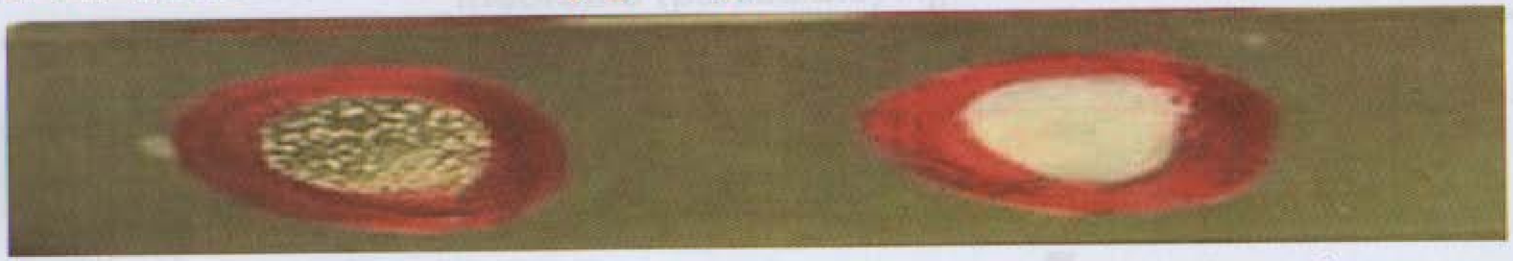
Slide Agglutination tests

Qualitative

Tube Agglutination tests

Quantitative

1-Identification and typing of isolated organisms



Test

(Saline + Organism + Antisera)

Granular Clumps (Positive)

Control

(Saline + Organism)

No Clumps

clumping in control???

2-Blood grouping

Tube Agglutination: Quantitative & Qualitative

Rack of Agglutination	*Widal Test*

1- Aim

Detect & measure level of **specific serum Abs**

2- Ab detected

Agglutinating Ab: IgM (mainly) & IgG

3- Ag used

Insoluble (particulate) Ag

4- Control tube

Ag + Saline

No agglutination ⇐ ⇒ *Agglutination*

↓ Normal Ag

↓ **Rough (autoagglutinable) Ag**

5- Uses

Rack of Agglutination	Widal Test
Diagnosis of **Brucellosis** **(Undulant fever)**	**1- Diagnosis of enteric fever** By: Salm.typhi , paratyphi A & B **2- Detection of post TAB vaccination**

6- Sample

Rack of Agglutination	Widal Test
Serum	**Serum from clotted blood**

Rack of Agglutination	***Widal test***

7- Steps

1- Serum

Is added in ***2 fold*** serial dilutions

1 row of 6 tubes	**4 rows each is formed of 5 tubes**
$\frac{1}{20}$ $\frac{1}{40}$ $\frac{1}{80}$ $\frac{1}{160}$ $\frac{1}{320}$ $\frac{1}{640}$	$\frac{1}{40}$ $\frac{1}{80}$ $\frac{1}{160}$ $\frac{1}{320}$ $\frac{1}{640}$

2-Antigen : added in constant amount

Brucella Ag	***a- Non specific Ag: in 1st row*** O Ag of 3 salmonella ***b- Specific Ags: Flagellar H*** Typhi 2nd row Para A 3rd row Para B 4th row

3- Incubation: 4 hrs at 37 C

4- Detect the Presence of agglutination

Identify **the titer in +ve cases:**

The maximum dilution of serum that still gives ***visible agglutination***

Interpretation of results

Rack of agglutination

1st tube (1/20)

No Agglutination

-ve case

Or

Prozone

phenomenon

Agglutination

+ ve case

Widal test

1-+ve case

Agglutination in :
O row & **1 H** row

Titre
> 80

2-Post TAB vaccination

Agglutination in:
O row & **3 H** rows

Titre
80

Scheme

Look to ***3rd*** tube (***1/160***) in ***O*** row

+ve agglutination

Case

Look to **4th** tube (**1/320**) in **O** row

to identify the ***O titer***

Look to **3rd** tube (**1/160**) in each **H** row

to identify ***type of Salmonella***

Look to **4th** tube (**1/320**) in the **+ve H row**

to identify the ***H titer***

-ve agglutination

Post TAB vaccination

The **O** & **H** titer

are ***80***

Prozone phenomenon in Brucella

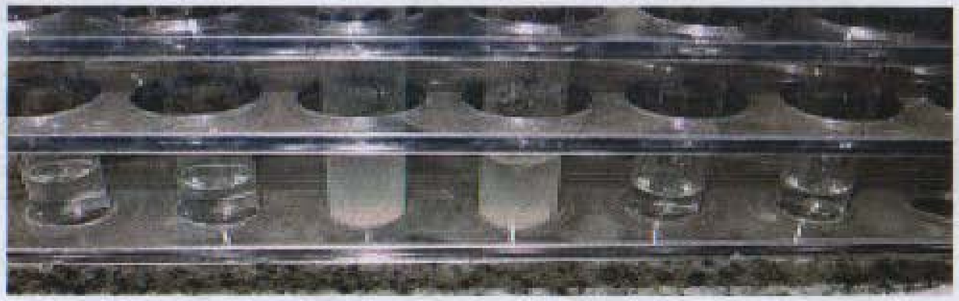
Definition

Absense of agglutination in tubes with **high conc.of Abs**

↓

False -ve result

If test is performed in **low n= of tubes**
(narrow range of dilutions)

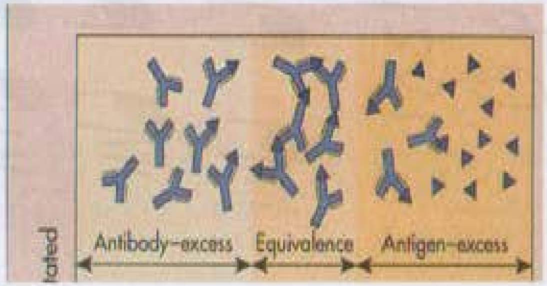


Causes

High Ab-Ag ratio

↓

No cross linking

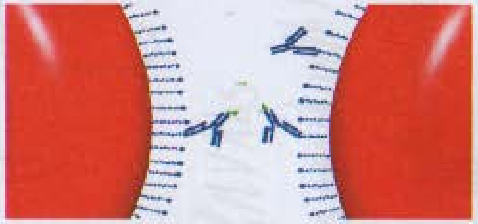


High level of **blocking (incomplete) Abs:**

Ig**A** or Ig**G**

↓

No cross linking



How to overcome this phenomenon?

Use **wide range** of dilution

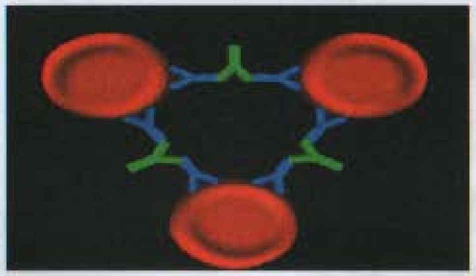
↓

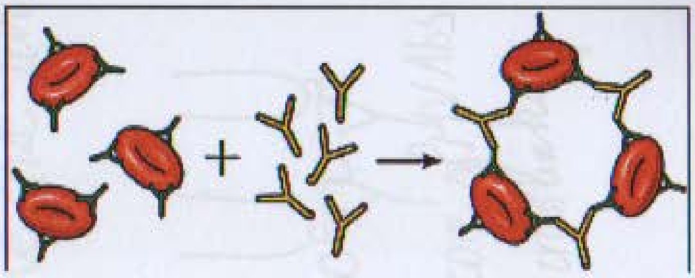
Overcome Ab excess

Add **Coomb's reagent** (Antihuman globulin)

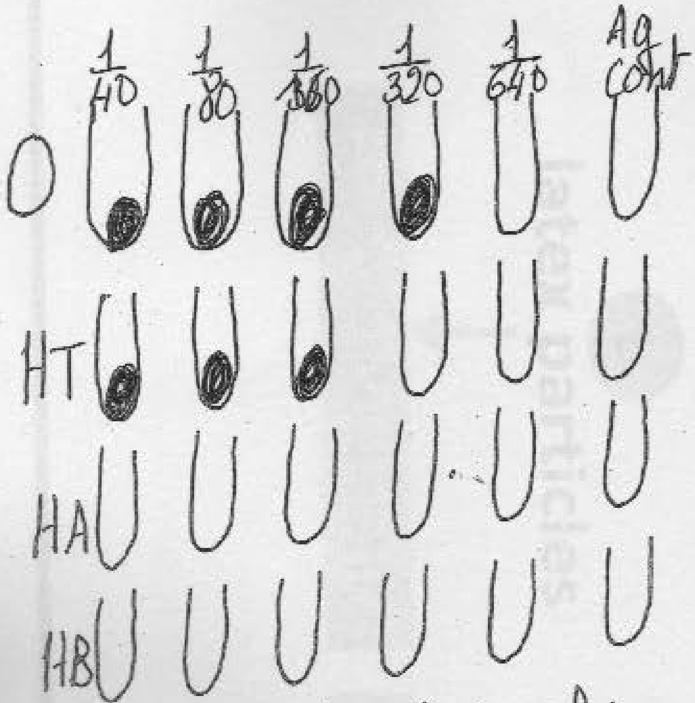
↓

Bind to ***Fc*** of ***blocking Ab*** → cross linking



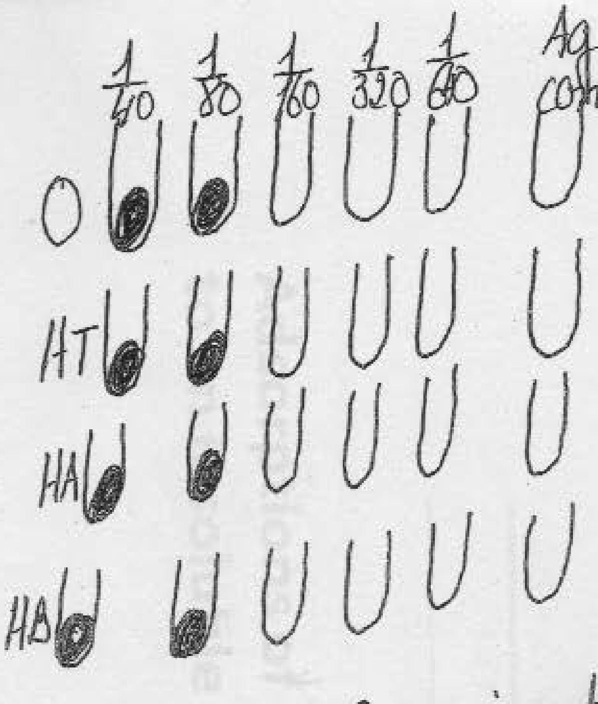


Widal Test



A case of Sal. typhi

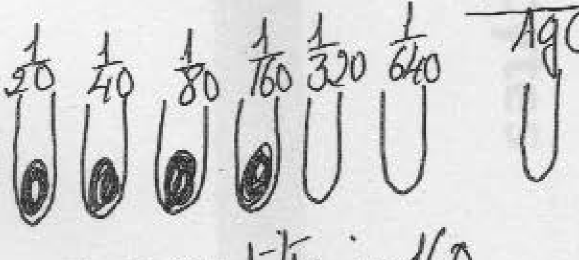
O titre: 320 HT: 160



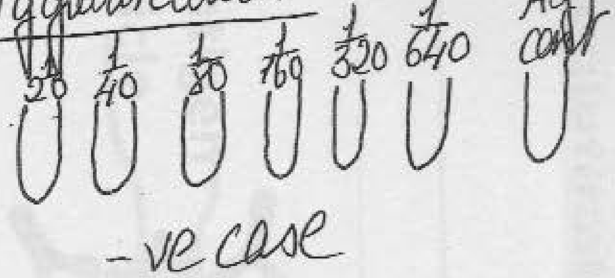
Post TAB vaccination

O & H titre:

Rack of agglutination

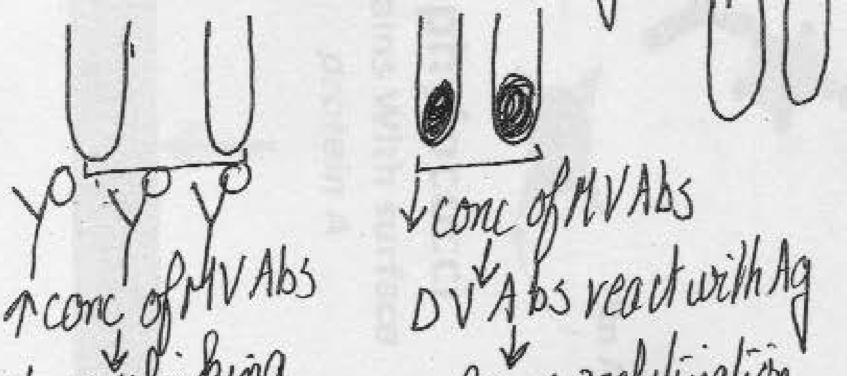


+ve case: titre is 160

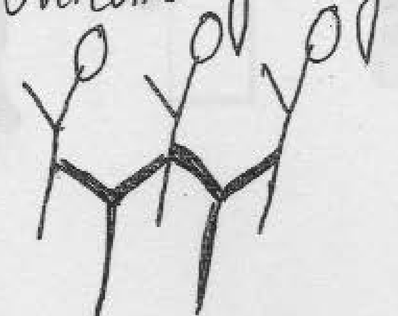


-ve case

Prozone phenomenon

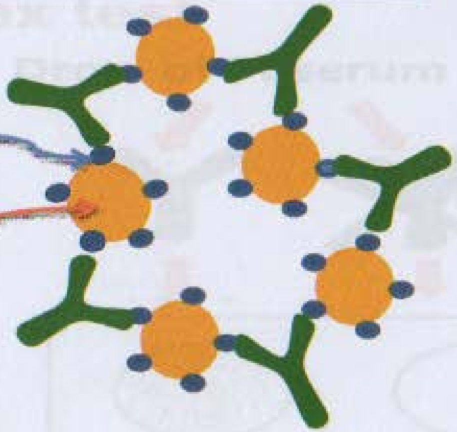


Overcome by adding Coomb's



Coomb's reag: Antihuman glob.
(Anti FC)

Passive (indirect)Agglutination tests



Adsorptions of soluble antigen to an insoluble inert particle

L

latex particles

↓

Latex agglutination

Erythrocytes

↓

Haemagglutination

Staphylococci

Strains With surface *protein A*

↓

Coagglutination

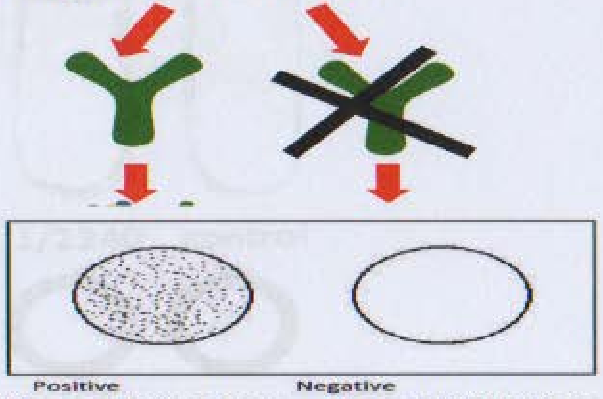
A-Passive Latex Agglutinations

Detection of antibodies against soluble toxins or enzymes of bacteria

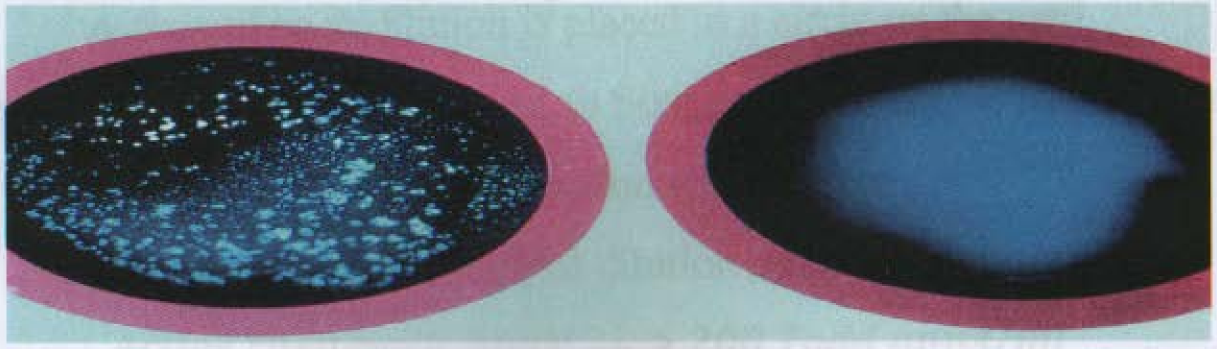
Antistreptolysin O test

1-Qualitative latex test

Drop of latex particles coated with soluble streptolysin O antigen + **Drop of Pt serum**

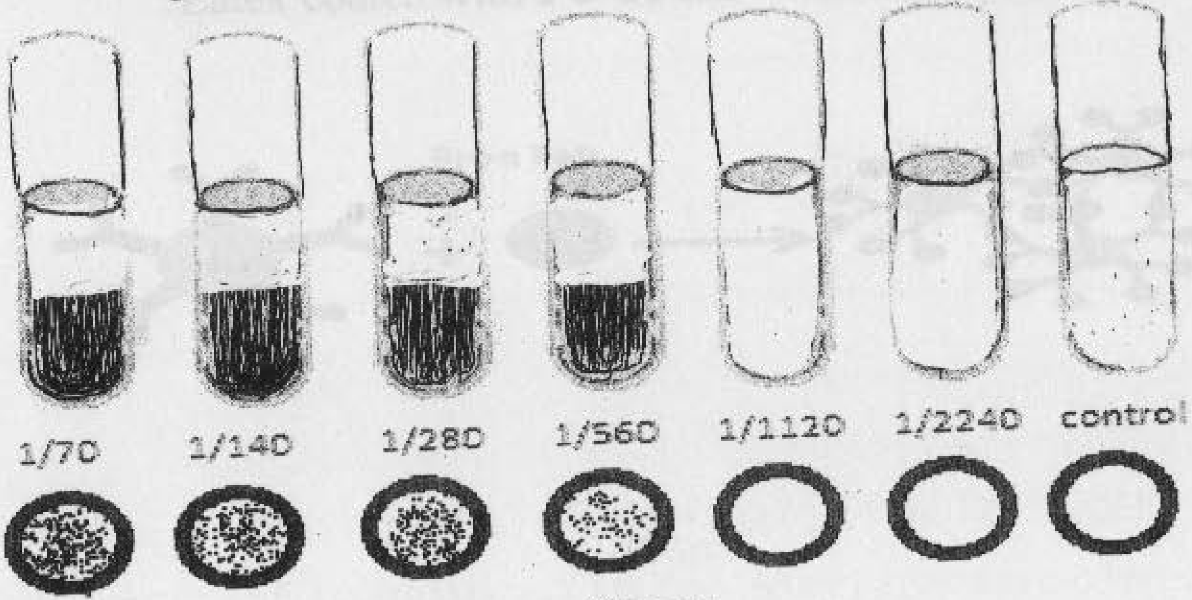


Visible agglutination **No agglutination**



Antistreptolysin O test

2_Quantitative Latex agglutination test



Steps

1-Serial dilutions of pt serum are made

⬇

A drop of each dilution is placed in a circle on the card

2-Reagent (Latex particle attached to Streptolysin 0 Ag) is added & mixed

⬇

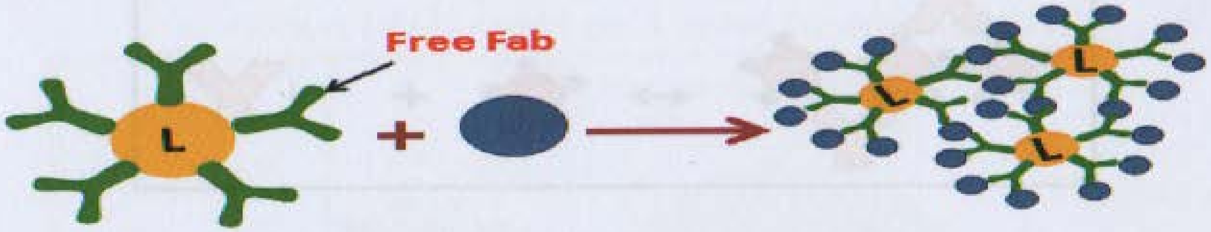
Visible agglutination within 2 min

3-Determine the titer: highest dilution showing agglutination

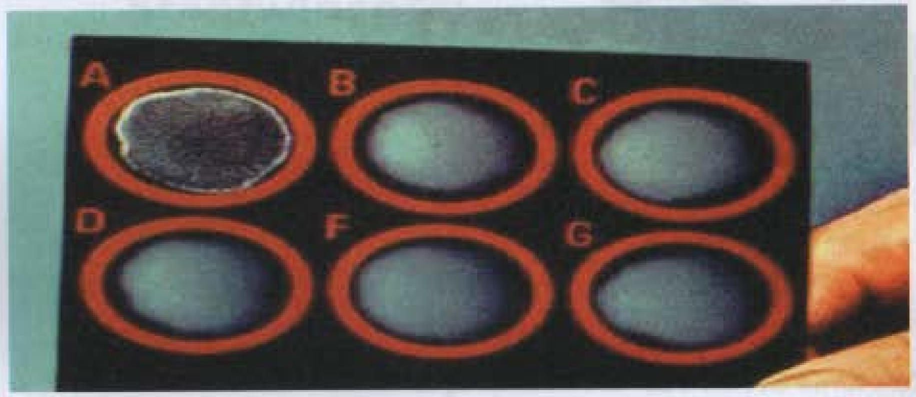
Active rheumatic fever → > 200 Todd units/ml

B-Reversed passive Latex Agglutinations

Latex coated with **Fc of Ab** ⟶ **detects Ag**



1-Lancefield Grouping of β hemolytic Streptococci

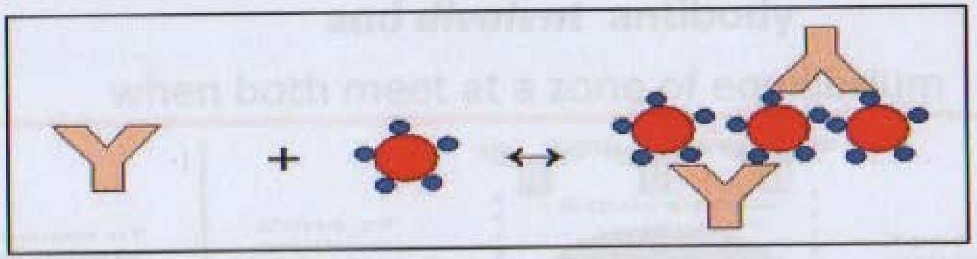


2-Detection of capsular Ag in meningitis

e.g Meningococci & Pneumococci

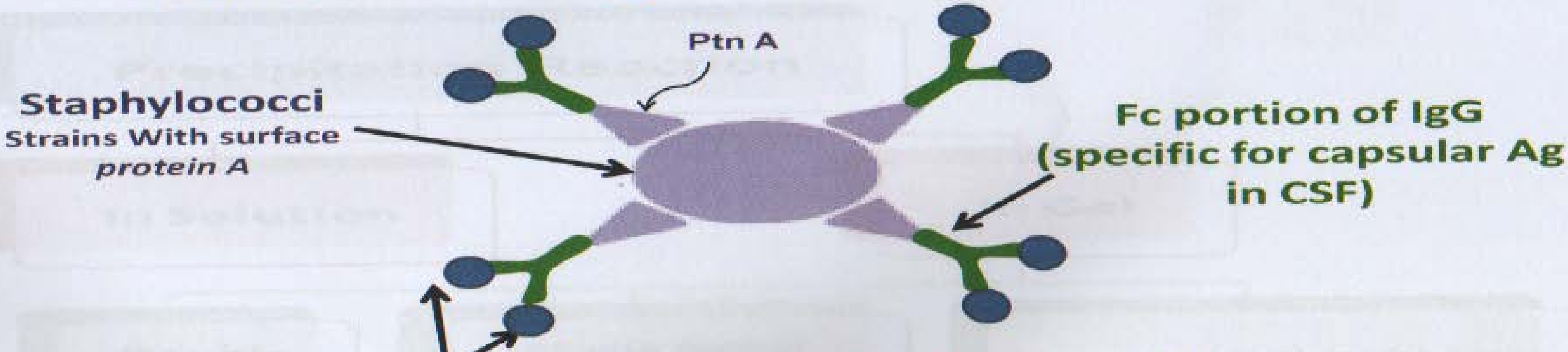
C- Passive Heamagglutination

RBCs coated with **soluble Ag** → ***Detects Abs***



D-Staph coagglutination

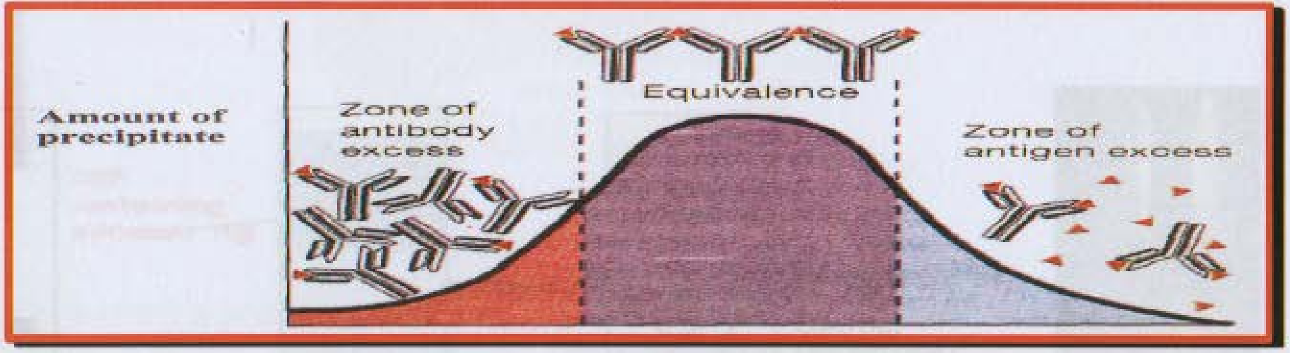
- Detects capsular Ag in CSF
- Diagnosis of **meningitis** by Meningococci&H.influenza



Capsule passively agglutinates with its specific antibodies carried on protein A

Precipitation

- Reaction between **soluble** antigen and ***divalent*** **antibody** when both meet at a zone of equilibrium

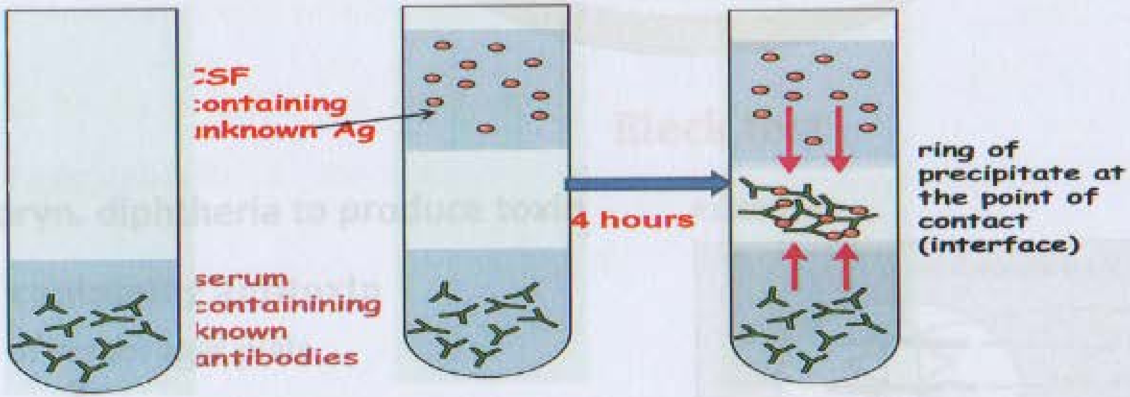


- **Precipitation Reaction**
  - **In Solution**
  - **In Gel**
    - **Double Diffusion**
    - **Single Radial Immune Diffusion**
    - **Immunoelectropheresis**

A-Precipitation in solution

1-Ring interfacial test

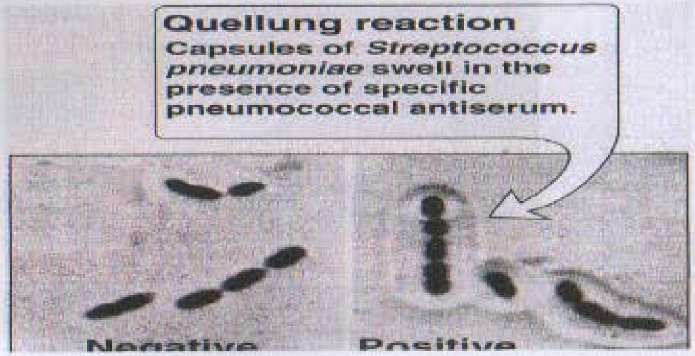
1-Detection of **capsule in CSF** → diagnosis of ***meningitis*** e.g Meningococci



2-Capsular swelling test :

Detection of **capsule in sputum**

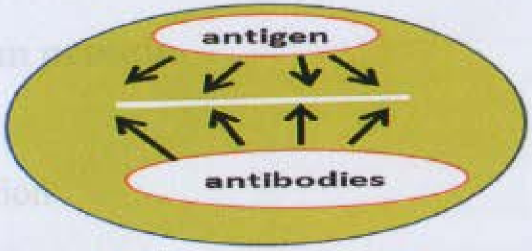
Diagnosis of ***pneumonia*** e.g Pneumococci & Klebsiella



Quellung reaction
Capsules of *Streptococcus pneumoniae* swell in the presence of specific pneumococcal antiserum.

B. Precipitation Reactions in Agarose Gels

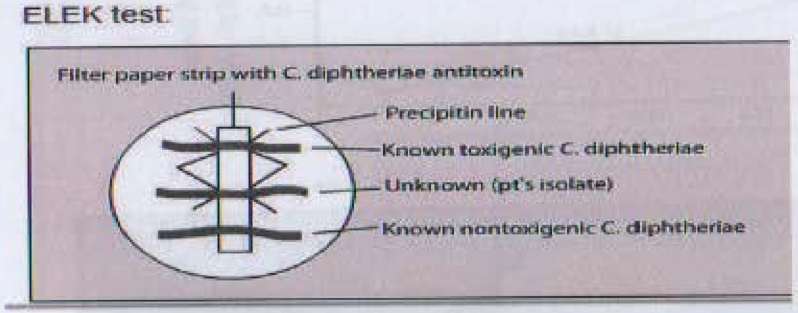
1- Double-diffusion method

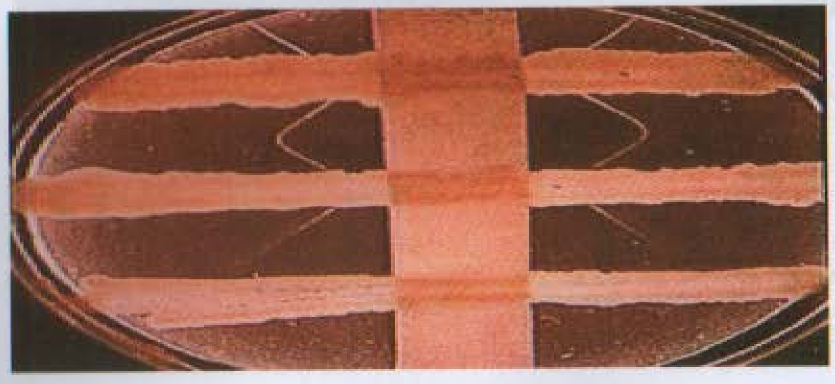


Eleck test

Aim: Ability of **Coryn. diphtheria to produce toxin**

Strip of filter paper **containing antitoxin**
is put in center of serum agar plate
⬇
Tested colony from ***throat of a carrier***
is cultured at right angle
⬇
If the org. is toxigenic,
the toxin will diffuse from the colony
⬇
Reacts with the **antitoxin**
which diffuses from the filter paper
⬇
Precipitin lines (within 18 - 48 hrs)


ELEK test:
Filter paper strip with C. diphtheriae antitoxin
Precipitin line
Known toxigenic C. diphtheriae
Unknown (pt's isolate)
Known nontoxigenic C. diphtheriae



2-Single radial immunodiffusion

Uses

Measure concentrations of **serum proteins** e.g

i.Complement components **ii.Ig** isotypes

Known Abs against the tested serum protein

are distributed uniformly

in agar solution during preparation

⬇

Add serum of the pt with unknown Ags

(serum protein) in one well

⬇

Add known **different concentrations of tested Ag**

in other wells

⬇

Diameter of ppt ring is proportional

to concentration of Ag

⬇

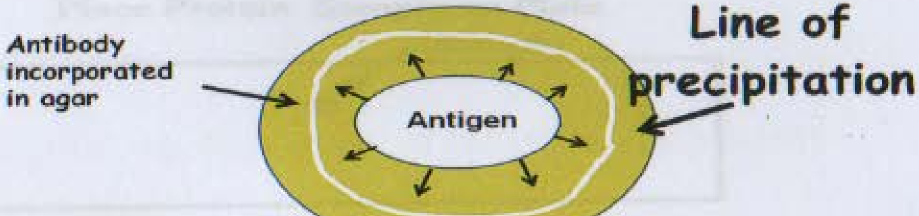
Compare the diameter of ppt ring

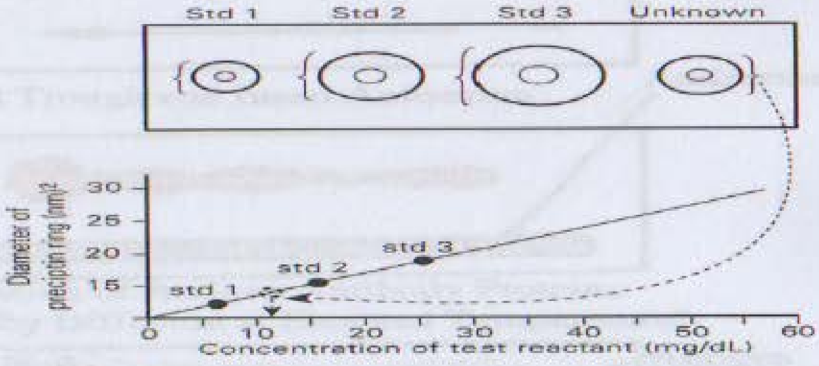
around the ***unknown well with other wells***

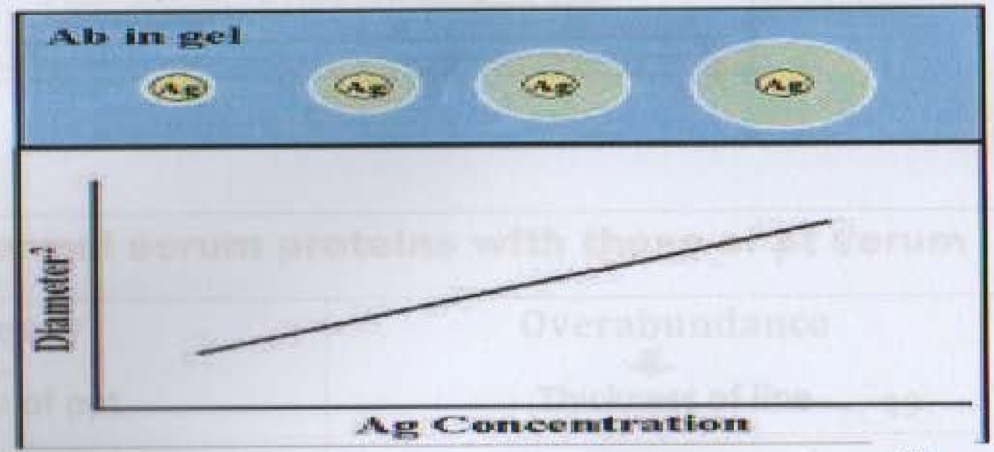
⬇

The unknown concentration

can be calculated **from standard curve**







3-Immunoelectrophoresis

Applications

Detection of **abnormalities**

in human serum proteins

in comparison with normal serum

Procedure

Pt serum is placed in a **well punched out in agar gel**

↓

Proteins are separated according to *their MW& charge by electrophoresis*

↓

A **trough** is cut in the gel

↓

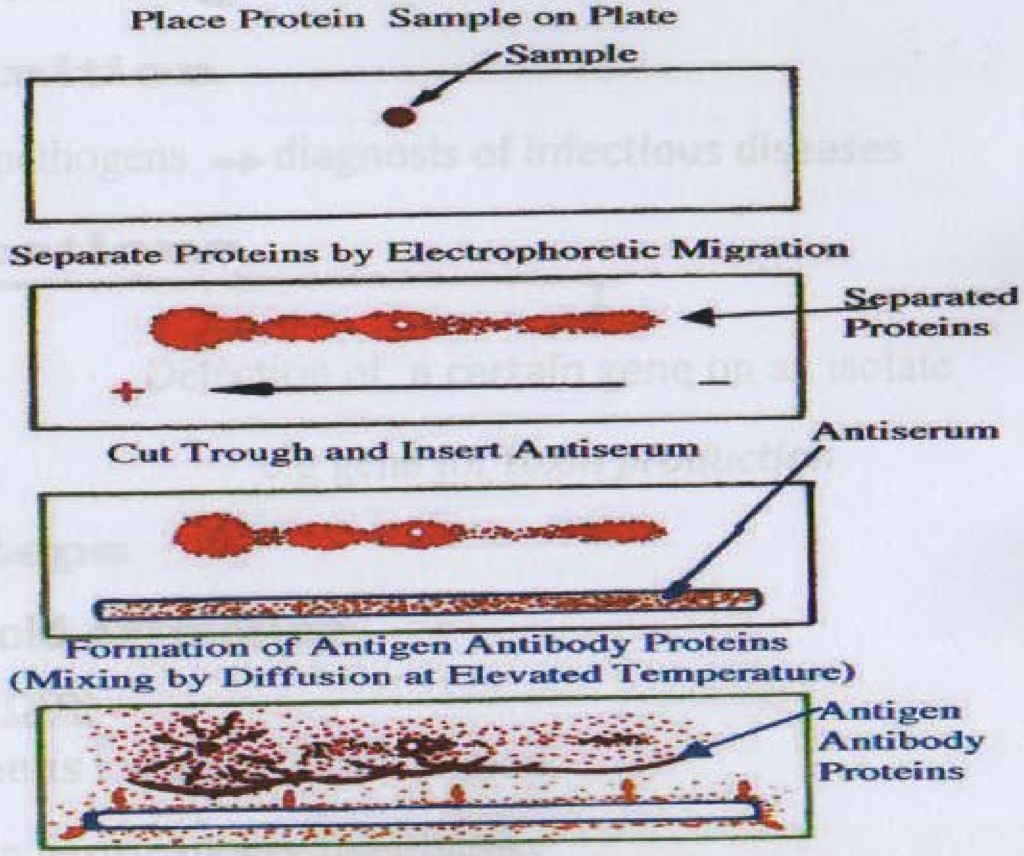
Abs to human serum proteins are placed in the trough

↓

Abs & separated serum proteins diffuse in agar

↓

Production of precipitin lines where Ag Ab reaction occurs


Place Protein Sample on Plate
Sample
Separate Proteins by Electrophoretic Migration
Separated Proteins
+
–
Cut Trough and Insert Antiserum
Antiserum
Formation of Antigen Antibody Proteins
(Mixing by Diffusion at Elevated Temperature)
Antigen Antibody Proteins

✓ **Compare pattern &intensity of lines of normal serum proteins with those of pt serum**

Absence	Deficiency	Overabundance
↓	↓	↓
No line of ppt	**Weak line of ppt**	**Thickness of line**

Molecular diagnosis

Definition

Techniques used to detect **nucleic acids** of pathogens → **diagnosis of infectious diseases**

Indications

Detection of org.**difficult or impossible to grow**

Detection of **a certain gene** on an isolate

e.g gene for ***toxin production***

Steps

A-Nucleic acid extraction

From:

1-Pathological specimens : sputum , urine , blood

2-Infected tissues : skin,gastric biopsy,liver biopsy

3-Bacterial colonies isolated on any culture media

B-Nucleic acid detection

A-Hybridization reaction

B-Polymerase chain reaction

Hybridization reactions

Probe : ***short*** segment of ***DNA*** or ***RNA complementary*** to that found in the detected org.

Denaturation ⟶ **Add probe Labeled with**

Org.in specimen is subjected **to high temperature**
⬇
Separation of 2 strands

- ♦ Enzyme
- ♦ Fluorescent dye
- ♦Radioisotope

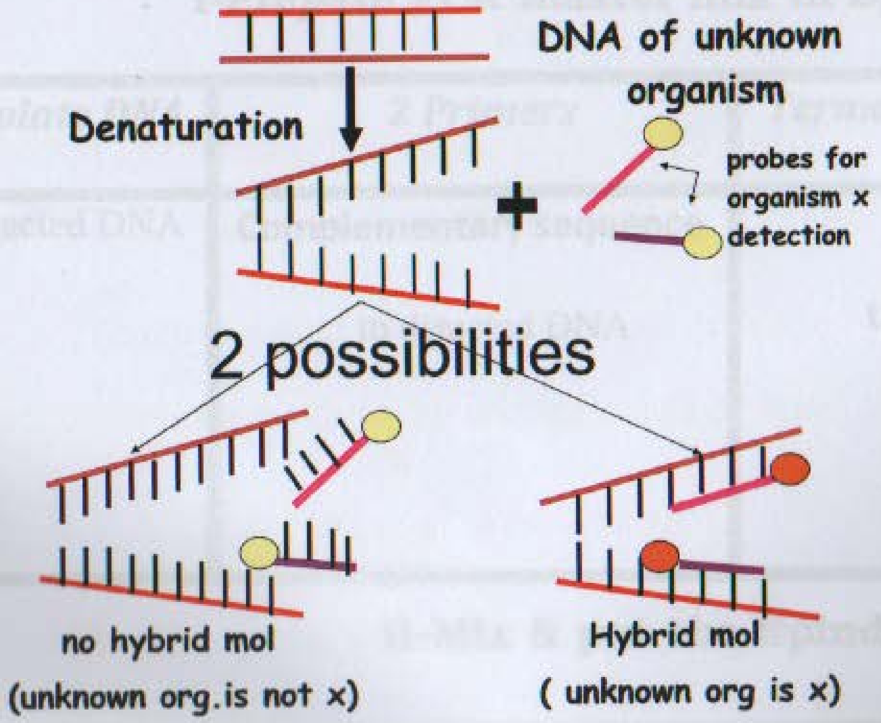
Uses

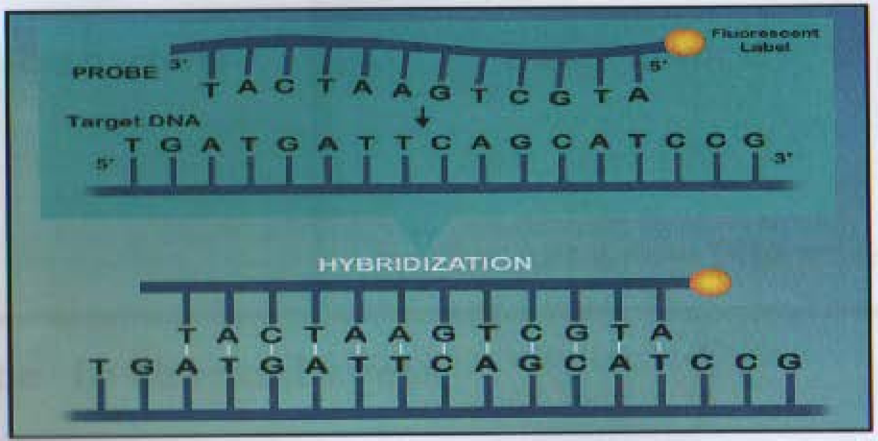
Rapid detection & identification of org

It is **complementary** to specific sequence **(finger print)** in the target org.
⬇
Bind to it
⬇
Labeled hybrid molecule

Specificity

- **Genus** specific
- **Species** specific
- **Strain** specific





Polymerase chain reaction

Principle

Amplify specific DNA sequence up to **10^6 folds**

⬇

Detection of very low quantities of org in clinical samples

⬇

Highly sensitive

Procedure

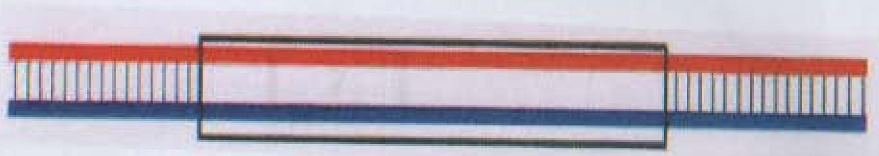
I-Prepare PCR master mix in Epindorf tube : Master mix contains

Template DNA	*2 Primers*	*Termostable DNA polymerase*	*4 nucleosides triphosphate*
Extracted DNA	**Complementary sequence** to detected DNA	Isolated from **thermostable bacteria**	♦ATP ♦TTP ♦GTP ♦CTP **Building blocks** from which DNA pol. **synthesizes a new DNA srand**

II-Mix & put the Epindorf tube in thermal cycle

III-Repeated cycles of amplification in thermal cycler : each consists of *3 steps*

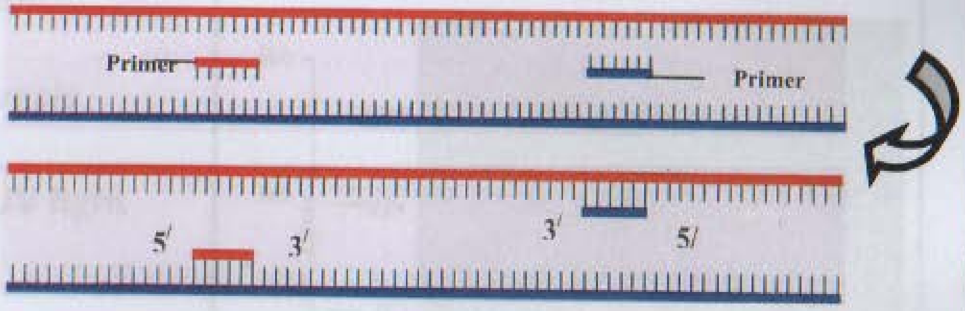
1- Denaturation : **Heating at 90- 95 C**



⬇

Separate double stranded DNA

2- Annealing : **Cooling to 45-60 C**



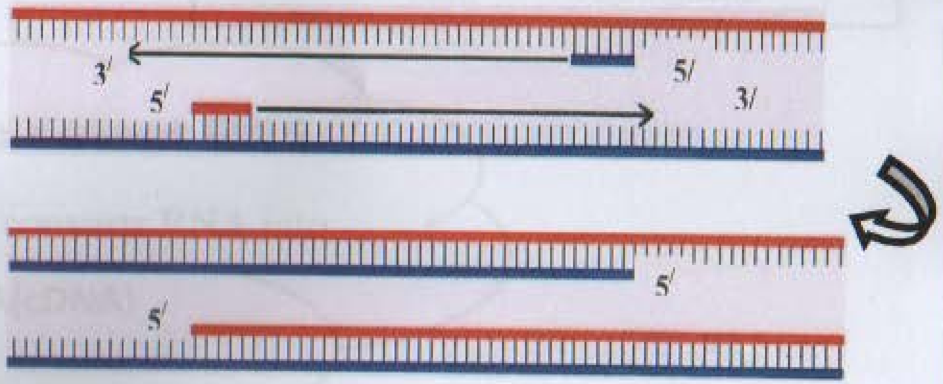
Add 2 primers : short DNA segments

complementary to the sequence to be amplified

⬇

Anneal to SS DNA

3- DNA synthesis (extension of primers) :



Temp is ↑ to 72 C

Thermostable DNA polymerase enzyme

extends each primer by **adding nucleotides**

⬇

Formation of new strand

⬇

Each cycle gives 2 folds amplifications

IV-Detection of amplified DNA target by :

A-Hybridization reaction

7

B-Agarose gel electrophoresis

PCR products are placed in **a well cut in agar gel**

⬇

Separated according to their MW by ***Electrophoresis***

⬇

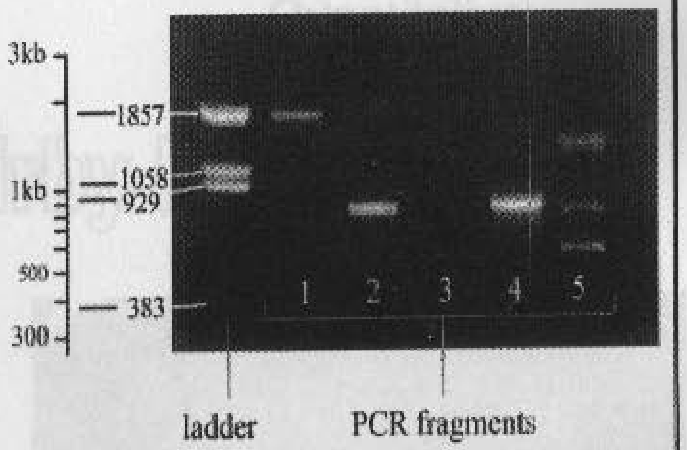
Stained with **dye** that **fluoresces** when exposed to **UV light**

⬇

Determine the size of PCR product:

By comparing it with that of ***size standard called ladder***

Verification of PCR product on agarose or separide gel



NB. RT-PCR is used to detect RNA

Reverse trancriptase enzyme converts RNA into

complementary DNA(cDNA)

⬇

cDNA is amplified

Antimicrobial susceptibility testing

DISC DIFFUSION
Qualitative

TUBE DILUTION
Quantitative

E TEST
Quantitative

I- Disc diffusion method (Kirby-Bauer)

Organism is cultured at standard conc.

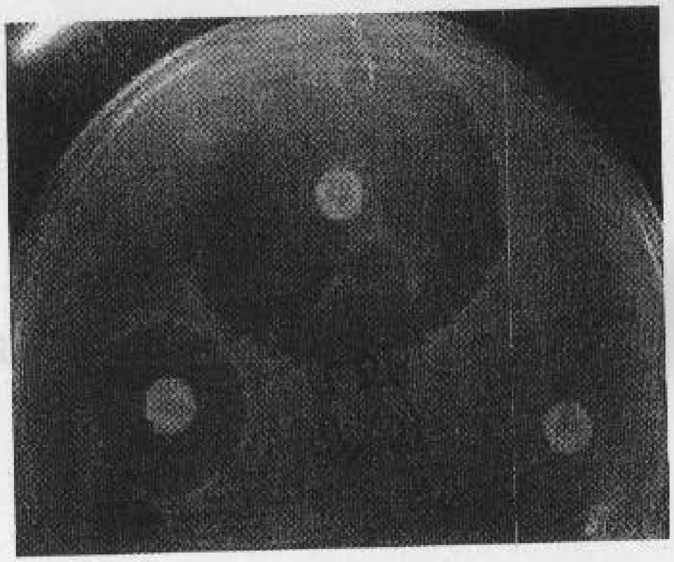
on **Muller – Hinton agar plate**

Add Filter paper discs

containing specified conc of different antibiotics

24-48 hrs

According to the diameter of zone of ⊖



Susceptible

Intermediate

Resistant

II-Tube dilution method

To determine

MIC (minimal ⊖ conc)

The **lowest conc.** of antimicrobial that ***prevents visible growth*** of org (no turbidity)

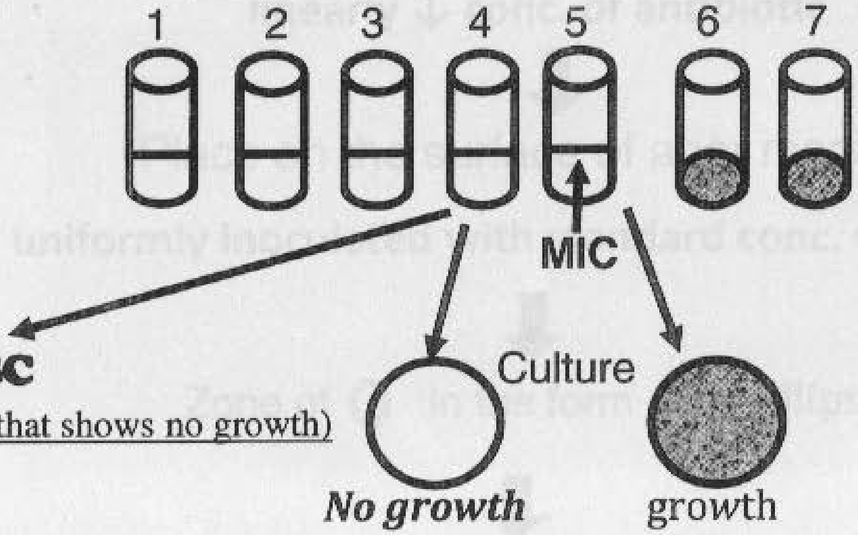
MBC (minimal bactericidal conc.)

The **lowest conc.** of antimicrobial that ***kills*** the org

Technique

Suitable fluid culture medium + known conc of org + **Serial dilutions of ↓conc. of antimicrobial**

Clear = no growth *Turbid* = growth



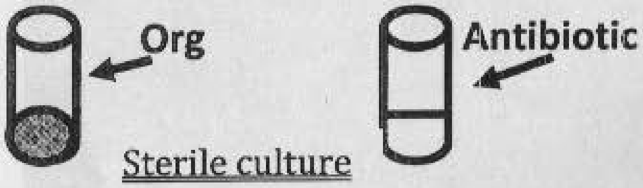
MBC

(highest dilution that shows no growth)

No growth growth

Culture cont

Antibiotic cont



Indicates viable tested org.

Indicates non contaminated antibiotic

Indications to determine MBC

- **Serious** inf. in **IS pts**
- Inf.located in a site **difficult to reach** with antibiotics

III- Epsilometer (E) test

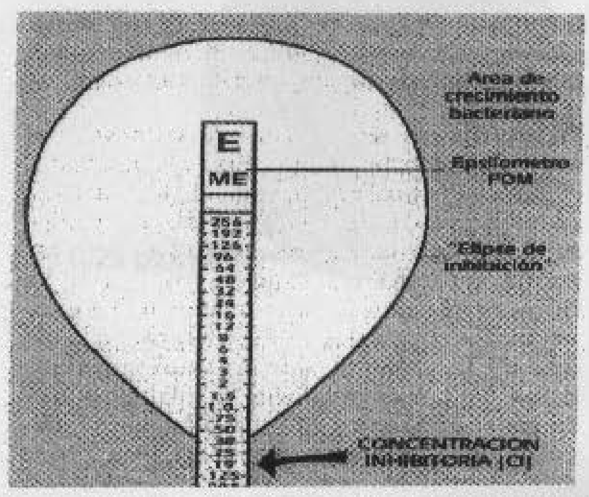
Modification of disc diffusion method

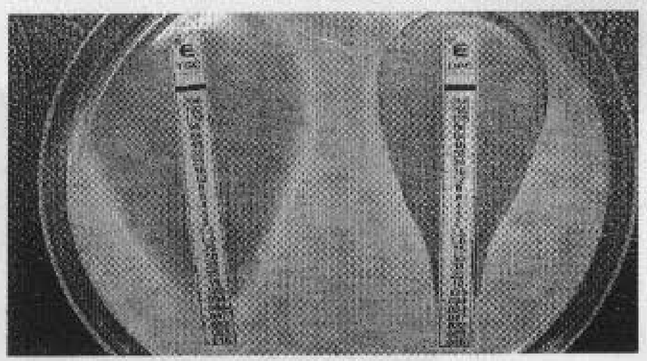
⬇

Determine potency of antibiotic + MIC

⬇

Quantitative


Area de crecimiento bacteriano
E
ME
Epsilómetro PDM
"Elipse de inhibición"
CONCENTRACION INHIBITORIA (CI)



Technique

Use multiple strips: Each strip contains

linearly ↓ conc. of antibiotic

⬇

Place on the surface of agar medium

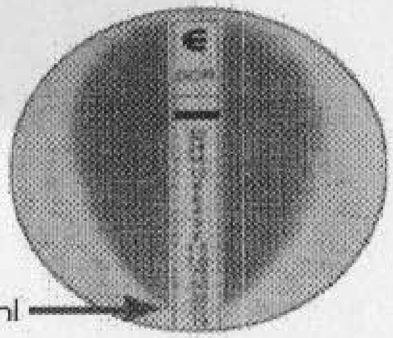
uniformly inoculated with standard conc. of test org

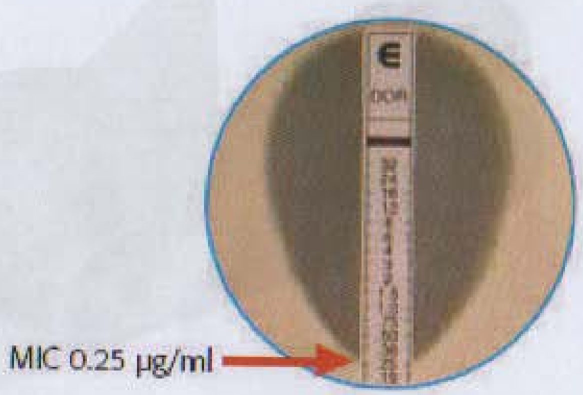
⬇

Zone of ⊖ in the form ○**of Ellipse**

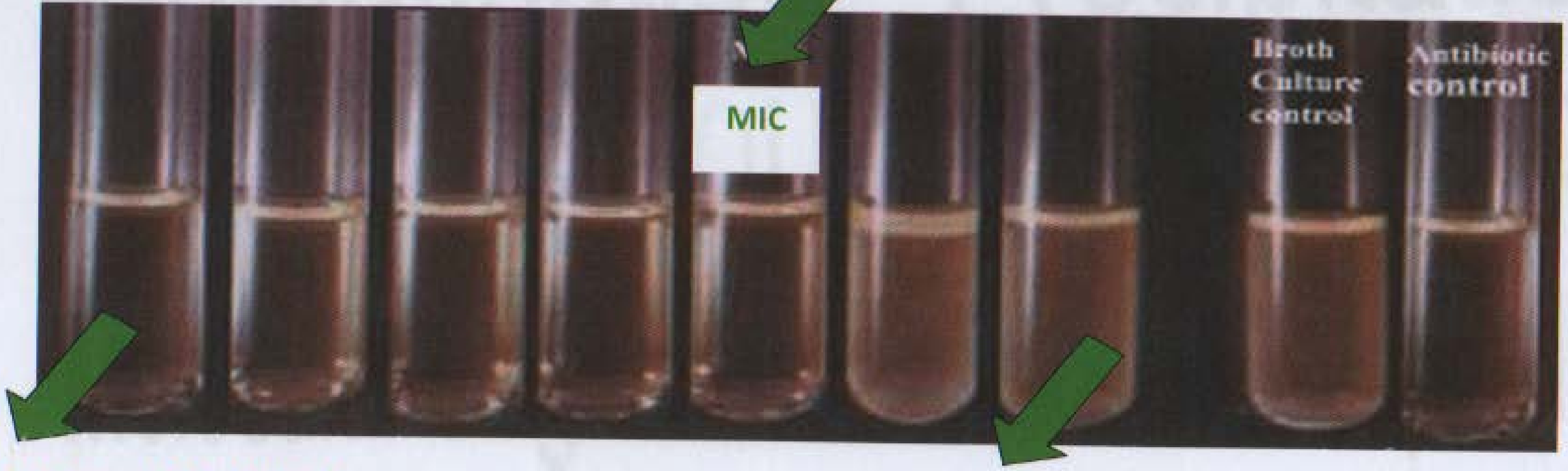
⬇

MIC is the point at which the ellipse meets the strip





MIC 0.25 μg/ml



MIC

Highest conc.of Antibiotic

Lowest conc.of AB